Results: Thirty-nine countries have agreed to participate (original target 22) and 29 countries have sent their preliminary data. Initial analysis suggests a relative paucity of CF patients in Europe who are over 35 years of age. Further analyses are underway to determine the reasons.
Conclusions: The project is on track to meet its milestones despite an unexpected rise in the take up from countries outside the European Union.

R18 OF MICE AND MEN: EARLY AIRWAY WALL DISEASE IN CYSTIC FIBROSIS

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The baby with cystic fibrosis (CF) has essentially normal lungs at birth, but at death has extensive airway wall destruction. This has been assumed to be secondary to infection and inflammation, but recent evidence has challenged this paradigm, and suggested that there may be a component of a specific CF related airway wall disease, independent of infection and inflammation. We initially showed that unscreened CF babies have airway obstruction at diagnosis, independent of any previous or present respiratory symptoms, signs and positive cultures, and this persists for six months, even despite intensive therapy [Lancet. 2001; 358:1964–5; Am J Respir Crit Care Med. 2002; 166: 1350–7 & 2004; 169: 928–33]. Recent data (Koslowska, unpublished) shows that even preschool children have CF related airflow obstruction, independent of infection. We explored this using endobronchial biopsy in CF children. We established the technique was safe [Pediatr Pulmonol. 2006; 41: 1021–4], and yielded acceptable material [Chest. 2007; 131: 1710–7]; we then showed that there were increased concentrations of elastin, glycosaminoglycans and collagen in CF BALF compared to controls, and each correlated with elastase activity, MMP-9 and neutrophil concentration. Both elastin concentration and MMP-9:TIMP-1 ratio, correlated negatively with FEV_1 ($r = -0.45$, $p < 0.05$ and $r = -0.47$, $p < 0.05$ respectively). Median reticular basement membrane (RBM) thickness was greater in CF (5.9 mm) than controls (4.0 mm, $p < 0.01$), and correlated with transforming growth factor-β1 (TGF-β1) concentration ($r = 0.53$, $p = 0.01$, but not with FEV_1. These data suggested there are two forms of airway remodelling in CF children: firstly, matrix breakdown which correlates with proteases and pulmonary function, and secondly RBM thickening, related to TGF-β1 concentration [Hilliard T, Thorax *in press*]. We supplemented these observations in the CF mouse nose [Hilliard T, Am J Resp Cell Mol Biol *in press*] by performing nasal lavage and serial coronal section through the nasal tissue of adult CF and wild type mice raised under normal housing conditions. Nasal tissue was also obtained from day 17 embryos and newborn pups. Detailed histological examination of the nasal cavity was performed. Bacterial culture, cell count and Macrophage Inflammatory Protein-2 (MIP-2) concentration were assessed in nasal lavage fluid. Significantly thickened respiratory epithelium and increased mucous cell density was found in adult CF mice of both mutations compared to wild type animals. There were no differences in bacterial growth, cell count or MIP-2 concentrations. Thus, there are structural and inflammatory changes in the nasal mucosa in adult CF mice, not present perinatally, that develop in the absence of increased lumenal bacterial infection. Hence evidence from both mice and men suggests there may be a CF-related airway wall disease, independant of infection, which may represent a novel therapeutic target.

R19 PHARMACOGENOMICS IN CYSTIC FIBROSIS

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Pharmacotherapy of cystic fibrosis (CF) basic defect, i.e. defective Cl^- secretion, requires a good knowledge of the loss of function mechanism associated with each CF mutation. Indeed, CF mutations are typically grouped in five classes according to the mechanism of action. Pharmacological approaches aimed directly at the CFTR protein have been designed for three classes of mutations. For class I, consisting in stop codon mutations, aminoglycoside antibiotics or the new agent PTC124 allow to produce an almost normal CFTR by a read-through mechanism. For class II, which includes F508del, pharmacological chaperones (also known as correctors) can be used to improve the maturation of the mutant protein. Finally, class III mutations, which cause a severe decrease in CFTR channel activity, can be treated with potentiators, small molecules able to restore the normal channel gating.
The specific mechanism of each compound will clearly require in the future a tailored use dependent on the CF patient genotype. However, the choice of a particular pharmacological approach will not only depend on the CFTR genotype but will need to consider also the influence of other genetic factors. For example, the efficacy of PTC124 and of other similar drugs affecting read-through of stop codon mutations may be limited by nonsense-mediated mRNA decay, a process that seems to be characterized by variability among individuals. On the other hand, pharmacological chaperones identified so far for F508del probably interact with cell proteins involved in CFTR biogenesis, intracellular transport, and degradation. Accordingly, individual variability in CFTR protein handling due to genetic factors may also affect the therapeutic outcome of F508del correctors.
Restoration of Cl^- secretion in CF patients may be also obtained with the so-called bypassing approach, i.e. by stimulating the activity of Ca^{2+}-dependent Cl^- channels. Denufosol, a drug that is now tested in clinical studies, activates Ca^{2+}-dependent Cl^- channels through interaction with the P2Y2 purinergic receptor. Also in this case, individual variability may affect the extent of therapeutic intervention. Indeed, it has been shown that polymorphisms in the P2Y2 receptor influence the intracellular Ca^{2+} response triggered by purinergic agonists and therefore the extent of Cl^- secretion.
As for other human diseases, the influence of genetic variability will need to be considered to optimize drug therapy in CF. This will be particularly important in the design and evaluation of clinical studies testing the efficacy of new drugs.

R20 FROM BENCH TO BEDSIDE: MODULATION OF AIRWAY INFLAMMATION IN CYSTIC FIBROSIS

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Neutrophilic airway inflammation is a characteristic feature of cystic fibrosis (CF) lung disease. There is ongoing controversy whether the CFTR mutation itself causes a pro-inflammatory milieu in the airways, which would imply that inflammation can precede infection, or whether inflammation is always secondary to infection. Studies in cell culture have yielded conflicting results. While initial studies showed increased NFκB activation and IL-8 production in unstimulated CF epithelial cells, others found a higher inflammatory response only after infection with P. aeruginosa. Animal models of CF do not exhibit the phenotype of chronic infection, but recent studies suggest that the response to infection is upregulated in CF mice. Furthermore sterile CF airways transplanted into SCID mice demonstrate higher IL-8 concentrations and luminal invasion of neutrophils, which would support the concept of a pro-inflammatory milieu in CF. While it is important to clarify this, there is sufficient evidence that airway inflammation is present in almost all patients with pulmonary manifestations of the disease. Since the presence of inflammation has been shown to be a risk factor for subsequent lung function decline, reliable tests to monitor airway inflammation in the clinic are urgently needed. Induced sputum is currently being assessed as a clinical and research tool, but unfortunately is not feasible in young children. While nonspecific treatment approaches that decrease infection or improve clearance of airway secretions were found to positively affect airway inflammation, specific anti-inflammatory treatment strategies have been less successful to date. A number of studies have now provided evidence that potent inhibitors of airway inflammation may potentially have a detrimental effect by promoting airway infection. This stresses the need to develop a better understanding of the factors regulating inflammation in the CF lung to develop better targeted treatment strategies in the future.

R21 EXPLOITING GENOMICS TO DEVELOP NEW ANTIBIOTICS AND VACCINES AGAINST CF-RELATED PATHOGENS

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Despite the development of potent antibiotics, *P. aeruginosa* and *S. aureus* still cause most of the morbidity and mortality in patients with cystic fibrosis (CF). Multiple courses of antibiotics in these patients have increased the resistance of the pathogens to many antibiotics and multi-resistant transmissible epidemic *P. aeruginosa* and methicillin-resistant *S. aureus* (MRSA) strains have been identified in CF centers. Sadly, the development of effective chemotherapeutic agents against these pathogens has ceased in the last decade. Thus, alternative approaches to develop antimicrobial agents with new modes of action and potent vaccines are needed. Genome sequencing and advances in post-genomic technologies such as functional genomics through antisense technology, insertion mutagenesis and bioinformatics analysis has opened the way for large-scale screening of bacterial targets for new antimicrobials. Integrated genomics, devised to screen whole bacterial genomes and to identify targets, different from those identified using conventional approaches can be used today. Using such techniques, key bacterial virulence determinants, expressed under host/pathogen conditions, providing essential functions for bacterial growth and pathogenesis *in vivo*, can be identified. To ensure broad protection, sequence variability and adaptive mechanisms, leading to gene mutations and homologous recombination have to be taken into account. Comparative genomic analysis is now considered as critical to provide a solid rationale to further proceed towards the development of antibacterials with global relevance.